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Platelet Function Testing

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INTRODUCTION

Many clinicians are challenged by a patient at risk for bleeding due to the presence of an acquired or functional platelet disorder. These complex disorders are difficult to diagnose in part due to the ambiguities in the laboratory assessment of platelet function. This review will discuss platelet functional activity and the traditional and newer laboratory methods for platelet function analysis.

PLATELET FUNCTION

The mechanisms by which platelets participate in hemostasis are complex. Extensive reviews are available to detail the mechanisms and pathology of platelet disorders. In brief, platelet activity can be divided into the following functions:

1) Adhesion-Platelets adhere to damaged blood vessels in a process mediated in part by binding of von Willebrand factor to the glycoprotein Ib-IX-V complex on the platelet plasma membrane.
2) Aggregation-This platelet-to-platelet interaction is initiated by many different agonists, which bind to specific receptors on the platelet membrane. *In vivo,* platelet aggregation is strongly dependent on fibrinogen binding to platelet GPIIb-IIIa.
3) Secretion-During activation and aggregation, the contents of platelet granules are secreted into the exterior environment where they play an important role in the augmentation and propagation of the hemostatic plug.
4) Activation of coagulation-platelets provide a procoagulant surface for activated coagulation protein complexes on their phospholipid membranes.

Platelet function studies measure and/or monitor the platelet's ability to adhere and aggregate. These tests have historically presented a challenge for the clinical laboratory due to the lack of reliable, accurate and easy-to-perform procedures.

PLATELET FUNCTION TESTING

Many methods have been used to measure platelet function. These include bleeding time, aggregometry, automated functional analyzers, thromboelastography (TEG) , and flow cytometric monitoring of platelet activation or adhesion markers.

Platelet aggregation is a measure of the *in vivo* ability of platelets to adhere to one another and form the primary hemostatic plug. It can be performed using either platelet-rich plasma (PRP) or whole blood. Substances such as collagen, ristocetin, arachidonic acid, serotonin, ADP, epinephrine, and thrombin stimulate platelets and hence induce aggregation. Response to these aggregating agents or agonists, provide a diagnostic signature for platelet function.

Platelet aggregation is affected by a number of confounding variables. Hemolysis complicates aggregation measurements since erythrocytes contain ADP; lipemic samples obscure spectral changes due to platelet aggregation and thrombocytopenia makes platelet aggregation evaluations difficult to interpret. This test is laborious and costly and is not readily available to all facilities.

Bleeding time was developed in the hope that quantifying the length of time a patient bled after a standardized incision would aid in the diagnosis of platelet disorders and would predict the risk of bleeding. Unfortunately, it is of limited assistance in evaluating individual patients, because of low reproducibility, questionable sensitivity, and unsuitability for serial testing and weak correlation to bleeding tendency. Despite these shortcomings, the bleeding time remains widely used in most community and tertiary care centers, although several alternative technologies for a platelet functional screening test have been developed.

The **Platelet Function Analyzer (PFA-100)** (Dade-Behring, Miami, FL) at present is the most widely used automated analyzer. This system uses membranes pre-coated with collagen/epinephrine or collagen/ADP to stimulate platelet aggregation. A blood sample is drawn under vacuum through a capillary that activates the platelets via shear force. The time it takes to form a platelet plug that blocks the aperture is measured and known as a closure time. Studies have demonstrated the utility of the PFA for the detection of acquired platelet disorders (aspirin induced bleeding) and von Willebrand's disease. The interest and use of the PFA-100 is increasing in the clinical laboratory due to the rapid, cost-effective and clinically relevant information that it provides.

Plateletwork's (Helena Laboratories, Beaumont, Texas) is a rapid platelet aggregation screening that is designed

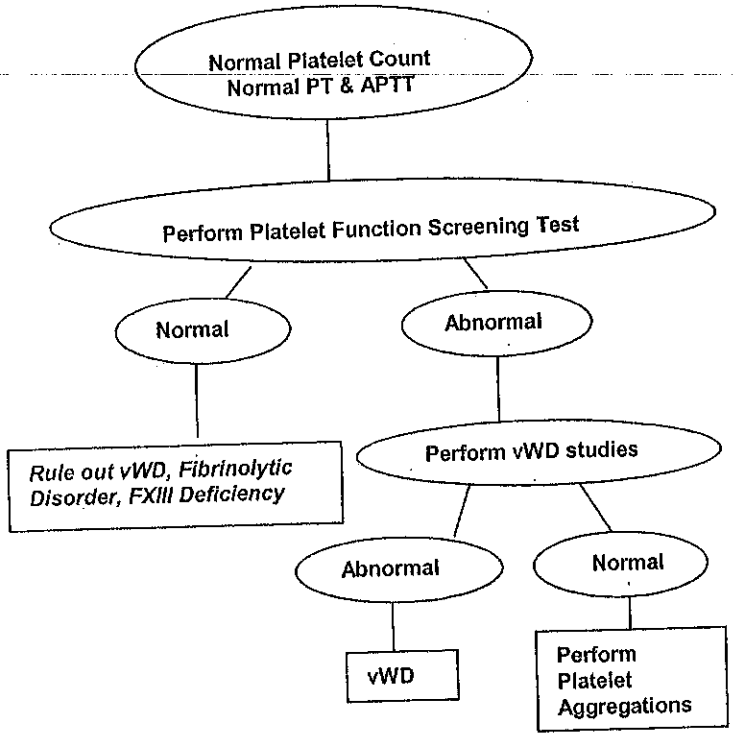
to determine the percentage of platelet aggregation in fresh whole blood samples taken during interventional cardiac procedures. It is a point of care test requiring a hematology analyzer and measures the change in platelet count due to aggregation of functional platelets.

Flow cytometry, long regarded as a research tool, has been gaining clinical use to study platelet structure and function. Methods have been developed to evaluate platelet activation and to diagnose platelet glycoprotein deficiencies. Cost and technically complexity has limited wide spread clinical acceptance of the method.

Other recent automated analyzers that have been introduced to evaluate platelet function are the Xylum Clot Signature Analyzer, the Ultegra RPFA and the HemoStatus test. In summary these analyzers provide different ways to evaluate platelet function and hemostasis. Experience with these instruments may provide complementary data to our existing platelet technologies to assist in the determination of the overall hemostatic status.

CLINICAL SUMMARY

Platelet dysfunction can lead to a clinical bleeding disorder that is congenital or acquired in nature. Laboratory evaluation of platelet dysfunction, although complicated, can be simplified. Prior to any laboratory testing a thorough medical, family, and drug history is essential in establishing the disorder, as is the exclusion of other coagulation or fibrinolytic disorders. The following outlines an approach for evaluating a patient with a suspected platelet dysfunction and a normal platelet count (the most common scenario).



CONCLUSION

Platelets have a seminal role in hemostasis and thrombosis. Platelet dysfunction may be acquired, inherited, or induced by platelet inhibiting agents. It is clinically important to assess platelet function to predict a bleeding risk, increase the understanding of the pathophysiology of platelet disorders and to provide more definitive patient management.

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